WEST AFRICAN COCOA RESEARCH INSTITUTE

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No. 44

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WEST AFRICAN COCOA RESEARCH INSTITUTE

T A F O

FORTY-FOURTH QUARTERLY REPORT : OCTOBER-DECEMBER, 1956

SENIOR STAFF

Director	J. Lamb (on leave 2nd December)
Acting Director	C.G. Johnson (arrived 24th December)
Secretary	H.L. Pugh
Director's Secretary/Librarian	Audrey Bryan

TAFO STATION

Division of Entomology	D.J. Taylor P.F. Entwistle Helen M. Entwistle
Division of Pathology	W.T. Dale A.L. Wharton G. Usher (on leave 5th December) A. Attafuah
Division of Botany	A.D. McKelvie (on leave 10th November) D.R. Glendinning R.G. Hurd (arrived 24th October)
Division of Soil Science and Chemistry	T.A. Rohan E. Margaret Holden P.W. Arnold R.K. Cunningham (arrived 21st November)
Division of Agronomy	R.J. Benstead (returned from leave 27th November)
Plantations Manager	H.W.S. Allison
Guide Demonstrator	F.E. Decker
Station Manager	S.E. Ntim Kwafo

IBADAN SUB-STATION

Entomologist (Acting Officer-in charge)	R.G. Donald (on leave 5th November)
Pathologist	J.M. Thresh

SYMPOSIUM ON CACAO BREEDING

A symposium on cacao breeding was held at Tafo from 1st to 4th October under the chairmanship of Dr. G.D.H. Bell, Director of the Plant Breeding Institute, Cambridge. It was attended by Mr. H.H. Croucher (Director of Agriculture, Western Region, Nigeria), Dr. J.M. Waterston (Director of Agricultural Research, Federation of Nigeria), Dr. W.R. Stanton (Maize Research Unit, Ibadan), Mr. H.H. Rogers (P.B.I., Cambridge), Dr. Walter Carter (Pineapple Research Institute, Hawaii), Mr. F.W. Cope (Senior Plant Breeder (Cocoa), I.C.T.A., Trinidad), Dr. G.R. Howat and Dr. B.D. Powell (Messrs. Cadbury Brothers), Messrs. A.E. Moss, P.S. Hammond, P.S. Castle and J. Bowden of the Gold Coast Department of Agriculture, and all the members of the senior staff at Tafo.

The first session was concerned with breeding for disease resistance with particular reference to swollen shoot disease. Mr. W.T. Dale summarized the available information about resistance and tolerance to swollen-shoot virus and Mr. A.L. Wharton gave a short paper on resistance to black-pod disease. At the second session Mr. A.D. McKelvie described the W.A.C.R.I. breeding material and afterwards a visit was made to the 8th and 9th Progeny Trials Areas. Mr. D.R. Glendinning gave a paper at the third session on 'Techniques of breeding: experimental considerations' and a paper by Mrs. P.F. Entwistle on 'Pollination of cacao' was read. At the fourth session a discussion on agricultural requirements as breeding characters was opened by Mr. H.H. Rogers. Manufacturers' requirements were considered at the final session with Dr. G.R. Howat giving the opening paper.

STAFF MATTERS

The Director flew to the United Kingdom on leave on 2nd December. Dr. C.G. Johnson, who has been seconded from Rothamsted Experimental Station for one tour as Senior Entomologist, arrived on 24th December and has assumed duty as Acting Director during Mr. Lamb's absence. Two other new members of the senior staff arrived during the quarter: Dr. R.G. Hurd and Mr. R.K. Cunningham who have been appointed Plant Physiologist and Soil Chemist respectively. Mr. Benstead returned from leave at the end of November and was posted to the Ibadan Sub-station to take over the duties of Officer-in-charge during Mr. Donald's absence on leave in South Africa.

Mr. Wharton visited Ibadan to have discussions with

the Plant Pathologist of the Federal Research Organization on a joint programme of work on strains of Phytophthora palmivora.

VISITORS

There have been even more visitors than usual at the Tafo station during the quarter. The Institute was honoured by a visit from the Prime Minister on 26th October. He was accompanied by a small party which included the Minister of Agriculture. Sir Gordon Hadow, the Officer Administering the Government, and Lady Hadow accompanied by Mr. R.K. Talbot, Regional Officer (Eastern Region) and Mrs. Talbot with Mr. S.J.E. Southgate, Chairman of the Managing Committee, visited the Institute on 14th December.

The Chairman and General Manager of the Cocoa Marketing Board visited the station on two occasions during November. On the first visit they came with four other members of the Board and on the second they accompanied the Minister of Trade and Labour. Other visitors have included Mr. Elysio Nunes, Director of the Cocoa Institute of Bahia, Brazil accompanied by the Swiss Consul, Accra; three members of the Ford Foundation, Professor W.O. Brown, Mr. M.J. Fox and Mr. J.B. Howard; Mr. P. Foster, Projects Officer (Cocoa) of the Gold Coast Agricultural Development Corporation; Dr. A.G. Forsdyke of the Meteorological Office, London, with Mr. H.O. Walker, Chief Meteorologist, Accra; Professor Lancelot Hogben; a party of members of the Western Regional Development Board, Nigeria.

Dr. G.R. Howat, Dr. B.D. Powell and Mr. G.A.R. Wood of Messrs. Cadbury Brothers spent several weeks at Tafo carrying out experiments on fermentation and drying.

Dr. Warren Weaver and Dr. R.S. Morrison of the Rockefeller Foundation and Mr. Woolaston of I.C.I. African Department visited the Ibadan Sub-station.

MANAGEMENT AND MAINTENANCE

There was again this quarter a small reduction of the labour force. Some of the maintenance labourers were transferred to cocoa picking during the peak season.

Five bungalows in the Residential Area were re-decorated and two of them had their verandahs mosquito-proofed. Minor repairs were carried out in W.A.C.R.I. village and a new door was fitted to the Recreation Centre.

A large number of trips by road to Ibadan were made to take cocoa pods to the Nigerian Departments of Agriculture.

The average daily production and consumption of water at the Tafo Station were as follows:-

	Production	Consumption
October	25,880 gallons	20,355 gallons
November	29,475 gallons	23,366 gallons
December	28,325 gallons	22,348 gallons

CROP DELIVERY

Weekly harvesting which began at the end of the last quarter has kept losses from diseased and damaged pods to insignificant levels. Crop records for the current quarter from the Old Station, Uniformity Trial areas are as follows:

TOTAL PODS		Pods discarded %	Pods fermented	Wet Wt. of beans lb.	Dry Wt. of beans lb.	Pods issued for experiments
Harvested	Discarded					
184,947	5,143	2.8	175447	37341.0	15858.7	4357

SWOLLEN SHOOT DISEASE

The numbers of trees removed during the quarter due to virus infection and other causes, are shown in the following table.

	OLD STATION			SQUARE MILE			
Month	Diseased	Other causes	Total	Diseased	Contacts	Suspects	Total
October	88	5	93	228	109	25	362
November	74	15	89	224	70	22	316
December	74	12	86	280	63	18	361
Total	236	32	268	732	242	65	1039

An additional 138 infected seedlings were rogued from the Old Station.

UPPER AMAZON SEED

The Regional Departments of Agriculture, Nigeria received a total of 12,758 F3 pods during the quarter.

METEOROLOGY

Month	1956		18-year mean	
	Rainfall (in.)	Wet days	Rainfall (in.)	Wet days
October	6.33	21	9.25	18.5
November	5.56	14	4.06	10.6
December	2.69	7	2.34	6.1
Total	14.58	42	15.65	35.2

VIRUS RESEARCH

Host range of Cacao viruses

Over the past two years of searching for natural infections in plants found to be susceptible to cacao viruses in laboratory tests, the following species have been tested, with negative results, in the numbers indicated:

Sterculia rhinopetala K. Schum. (198); *S. tragacantha* Lindl. (127); *Cola gigantea* var. *glabrescens* Brennan & Keay (21); *Hildegardia barteri* (Mast.) Kosterm. (19); *Bombax buonopozense* P. Beauv. (5).

These negative results may not mean that all the tested trees were healthy, because virus availability, even in artificially infected saplings of these species, soon falls to a very low level. There was one positive diagnosis in *Ceiba pentandra* Gaertn. during the same period (see Quarterly Report No. 41) in tests on 61 trees. Field testing has now been suspended, except for that of *Adansonia digitata* L., in which 16 infections have so far been found amongst over 1,000 specimens examined.

In laboratory tests, two plants of okra (*Hibiscus ?manihot* L.) seem to have been infected with the Kpeve virus; each gave a single positive 'back-test'. These and other plants colonized with infective mealybugs shed some of their leaves and one of the two apparently infected specimens died within a few weeks; but it is not clear whether the symptoms resulted from virus infection, mealybug feeding, or some other cause. Although the possibility of contamination seems unlikely, the test is being repeated. A similar experiment with New Juaben gave negative results. Material of *Ceiba* obtained from Indonesia by Prof. H.G. Baker has proved susceptible to this virus.

Transmission studies

Planococcus citri (Risso) has transmitted the

Adansonia virus (AD75) from Wasipe (Northern Territories) and Pseudococcus sp. near gahani Green the Bosomuoso (2) cacao virus.

Over 90% of mobile mealybugs (Pseudococcus njalensis Laing) on cacao trees are first-instar nymphs (Cornwell, Ann. Rep. WACRI, 1955-56). Interest, therefore, centres on whether these feed before or during their migrations and are thus capable of transmitting cacao viruses. Gravid females were placed for 24 hours on comparable young Amelonado seedlings infected by the New Juaben virus. The nymphs produced were transferred singly from the infected plants to indicator beans 0, 24 or 48 hours after the removal of their parents, separate plants being allocated to each treatment to avoid undue disturbance. Of 107 nymphs 0-1 days old on or near the source plant, only 12 appeared to be feeding at the time of their removal to indicator beans, and none transmitted infection. At 1-2 days old, 17 out of 47 of the insects appeared to be feeding, but transmission was effected only by four that were not. Obviously these had fed, and perhaps the 17 had not done so long enough to have a reasonable chance of acquiring virus. Twelve out of 25 nymphs 2-3 days old infected their indicator beans, and those apparently feeding seemed to be about as efficient as the remainder (8/17 transmissions as against 4/8). It is plain that first-instar nymphs of P. njalensis no more than 2-3 days old are efficient vectors of New Juaben, and in view of their mobility and numerical preponderance insects of this stage must play an important part in its natural spread.

Previous workers have reported transmission of cacao viruses from pods, but no published record of the latters' state of maturity is known to the writer. Because only ripe pods are normally transported, and these are often colonized by mealybugs, it is important to know whether virus can be transmitted from them. It was considered that the senescent shells of ripe pods might contain little or no available virus, but this is not so; using ten nymphs of P. njalensis per indicator bean, transmission rates of 8/11 and 4/8 were obtained from completely green and fully ripe pods from infected trees. After these results had been obtained, the writer's attention was drawn to unpublished data collected by Dr. T.W. Tinsley. Using ten adults or 30 nymphs per indicator bean, he obtained transmission rates of 8/12, 20/23 and 42/44 from immature green, fully grown but unripe and ripe pods, respectively.

Viruses and virus strains

In protection tests, the Nigerian virus from Asalu showed no close relationship to Alaparun (also from Nigeria), three isolates from A. digitata (AD7, 36 and 135), or one from Cola chlamydantha K. Schum. (Anibil). Similarly, mild New Juaben gave no protection against the last-named and three other C. chlamydantha isolates (Aboboya, Adiembra and Aiyim).

Observations on the relative virulence of selected Gold Coast and Nigerian isolates towards Amelonado cacao (see Quarterly Reports No. 41 & 42) are being continued. Five plants per replicate are infected (as dissected beans) with each isolate; two replicates have been completed (the plants being 'harvested' at eight months old) and others are in progress. Certain observations on symptoms and growth are made during the life of the plants, and records taken after 'harvesting' include the following: plant height; number of leaves present and shed; leaf products (length x breadth); fresh and dry weights of entire plants and their component parts (leaves, stem, tap root, lateral roots).

Only a few general comments based on the first two replicates are made here, since it is proposed to prepare a full account when the experiment is complete. The virulent New Juaben virus reduced total dry weight to only 16% of that for healthy controls, and some plants died. Corresponding figures for the other viruses were Bosomuoso (2) (W.P.) 28%, Asijere (Nigeria) 30%, Ilesha (Nigeria) 39%, Kpeve (Togoland) 43%, Aiyiboso (W.P.) 45%, Offa Igbo (Nigeria) 52%, Olanla (Nigeria) 62%, Mampong (E.P.) 69%, Bosumtwe (Ashanti) 73%, Amafie (W.P.) 76%, Wiasi (W.P.) and Bisa (Trans-Volta) 81%. There was reasonable agreement between the replicates; except for Kpeve, which rose from sixth in order of virulence in the first replicate to third in the second, no isolate changed its position by more than two places. Kpeve seems to owe much of its severity to excessive defoliation, an average of over 18 leaves per plant being lost. This figure exceeds even that for New Juaben (15). In plants infected by the latter, absorbing roots are almost non-existent (as reported by Goodall, Ann. appl. Biol. 36, p.440, 1949). Bosomuoso (2) and Asijere also have a serious though less pronounced effect on the lateral roots.

Resistance and tolerance

In a series of experiments using as sources of the virulent New Juaben virus two-year-old Iquitos-type seedlings

with symptoms of varying intensity, and one young adult of P. njalensis per indicator bean, the following transmission rates were obtained: severe, 38/215 (17.7%); intermediate, 30/282 (10.6%); regular mild symptoms, 21/145 (14.5%); very mild sporadic symptoms 1/248 (0.4%). Plants in the first three categories gave very variable rates of transmission. For severely affected plants this is understandable, because sometimes only rather woody stem apices were available for mealybug feeding and such tissues contain relatively little transmissible virus (Posnette & Robertson, Ann. appl. Biol., 37, p.363, 1950.) From the results obtained with seedlings showing only sporadic symptoms, it seems that highly tolerant plants contain very little available virus. If confirmed by more extensive tests this may have important practical implications.

Of six Iquitos-type 'twinned' plants (Quarterly Report No. 36, et seq.) all of which developed mild evanescent symptoms after infection with the same virus, two have given no further evidence of infection after cutting back to provide material for rooted cuttings (loc. cit., No. 42); nor have the latter shown infection by symptoms or 'back-tests' with vectors. Cuttings from the other four plants have reacted variably: those from one have all shown symptoms and given positive 'back-tests'; two plants have yielded both symptom-bearing and symptomless cuttings, and one of the latter has given no evidence of infection by 'back-testing'; of four cuttings from the remaining plant, which has itself shown symptoms since coppicing, none has yet given any indication of infection. In the light of results described in the preceding paragraph, negative 'back-tests' in this experiment cannot be taken as evidence for the absence of virus. It is proposed to test the doubtful cuttings for infection by budding them with Amelonado material. All patch grafts taken from four mildly affected Nanay-type trees (loc. cit.) have produced severe symptoms in the Amelonado stocks.

The third and fourth replicates of the experiment to compare resistance and tolerance to virulent New Juaben in 50 F1 Iquitos-type (T17) seedling trees (Quarterly Report No. 38, et seq.) are still not quite complete, due to a shortage of material suitable for cuttings from some stands. For every clone, all five cuttings have been infected in at least one replicate, so there is no evidence of marked resistance. The use of 50 P. njalensis nymphs per plant has proved to be a

mistake; greater differences between the clones might have been revealed by using smaller numbers. Amongst the more promising stands (having regard to the total number of uninfected cuttings and their distribution amongst the replicates) are : T17/2652, 448, 1159 and 2655.

The similar experiment with Kpeve (loc. cit., No. 42) is continuing. It is already obvious that the Iquitos material is more resistant and tolerant to this virus than to virulent New Juaben.

Attempted mechanical transmission

Seedlings of Zinnia and Ipomoea (Scarlett O'Hara Morning Glory) have been used as test plants. Extracts from leaves of cacao seedlings infected with the New Juaben virus were made as in previous experiments with buffer solutions containing reducing agents. Gelatin was also added for making some extracts and it prevented the scorching which occurred when it was not included. There were no transmissions of virus.

Chemotherapy

An experiment which had been started on the effect of thiouracil on infection with swollen shoot virus was described in Quarterly Report No. 43. The seedlings which were watered daily with a solution of thiouracil after being bombarded with mealybugs from a virus source, and others which were treated with thiouracil both before and after bombardment, developed symptoms more slowly than untreated controls. Three months after the bombardment all the control plants showed severe symptoms of infection, but 6 out of the 40 thiouracil treated plants were still apparently healthy and some of the infected ones had only mild symptoms.

However, in another experiment, in which beans were bombarded and then watered daily with a 50 mg./l. solution of thiouracil, the treated seedlings developed symptoms at the same rate as the untreated controls and the symptoms were more severe. The apparent difference between the effects on infection in the bean and seedling stages is being further investigated.

STUDIES ON BLACK-POD DISEASE

Fungicidal control trials

Trial I will end in January, 1957; levels of pod-disease incidence were low during the current quarter, for which records are given below in an abbreviated form:-

Pod yields - Trial I

Fungicide	Mean yield per tree	Damaged %*	'Black pods' %*	Rodent damaged %*	Otherwise damaged %*
Unsprayed (Control)	22.2	24.8	8.6	9.9	6.3
Carbide-Bordeaux mixture	16.5	20.6	3.5	13.9	3.3
Ordinary Bordeaux mixture	17.5	21.0	5.5	12.6	2.8
Perenox	18.8	20.8	3.4	14.1	3.4

*Classified pods as a percentage of mean yield.

Unlike last quarter, the F2 frequency of spraying has given the least control of black-pod disease, indicating that there has been little residual effect from the eight fortnightly applications during the period May to August.

Cushion infection

On the second 'pod-painting' experiment (Quarterly Report No. 42) the occurrence of only a few 'black pods' in relation to the large numbers of healthy ripe pods harvested during the quarter has rendered the season's results of little value.

Effect of age and size of pod on susceptibility

Nearly all the labelled fruits have been harvested from the 200 trees included in this experiment. It is intended to continue these studies, and labelling of cherelles for the 1957/58 season has started.

Strains of Phytophthora palmivora Butl. occurring in Nigeria and the Gold Coast

The Commonwealth Mycological Institute at Kew has co-operated in preliminary investigations into the growth and pathogenicity of P. palmivora isolates from Nigeria and the Gold Coast. The first series of tests there suggested that isolates sent by the Pathologist of the Federal Department of Agricultural Research in Nigeria differed markedly from those sent from the Gold Coast. In a second series of tests, difficulty was experienced with the technique of inoculating cacao pods, which casts doubt on the validity of the initial results. It is not yet known to what extent the isolates differ with regard to

growth characteristics and morphology.

A joint programme of work has been drawn up with the Pathologist of the Federal Department of Agricultural Research to study Nigerian and Gold Coast isolates in their respective territories.

CAPSID STUDIES

Population studies

The experiments, first set up in January 1956, to investigate the possibilities of estimating the capsid population by egg counts are continuing.

Vertical distrubition of Capsid eggs

Capsid populations having reached a suitably high level, sampling has been commenced in the plot of mature cacao thinned in the previous quarter from its original 4' x 4' spacing. Should the results prove that there is a significant change in the vertical distribution of capsid eggs with wider tree spacing (and its resultant effect on light intensities within the canopy) then the plot will be further thinned to obtain an even wider tree spacing.

Capsid rearing

This work has been held up recently due to a shortage of cherelles, though it has been found that cut pieces of green pods serve almost equally well for feeding and oviposition, when they are kept in an atmosphere of one hundred per cent humidity.

Investigation of fungi associated with Capsid lesions

Dissemination

Nine hundred and ninety-six insects belonging to 64 families have been examined for the presence of Calonectria rigidiuscula (Berk. & Br.) Sacc. (Fusarium decemcellulare Brick), and 22 in 13 families have been found contaminated with it. The members of 10 of these families are phytophagous. Investigation of the internal fungal flora of capsids failed to show the presence of C. rigidiuscula, and measurement of the internal diameter of their stylets confirmed that it is too small to allow conidia of the fungus to pass. The occasional presence of C. rigidiuscula in the soil was demonstrated by introducing sterilized cotton-wool plugs soaked in cassava-2%dextrose agar; 1.1% of the tests were positive. Rain-water collected in sterilized milk-bottles under a capsid-damaged tree showed the presence of C. rigidiuscula when plated on cassava-dextrose agar.

Factors affecting host reaction

When pieces of cassava-dextrose agar with actively growing and sporulating F. decemcellulare were placed in contact with the roots of transplanted cacao seedlings in boxes of soil, 50% had completely wilted in a week, compared with none of the control. There was no sign of mycelium or internal damage in the roots or shoot of the wilted plants, and the fungus could not be isolated from them. Thus, it is likely that the wilting was due to absorption of toxins from the agar rather than direct attack by the fungus. F. decemcellulare could not be isolated from soil in the boxes after three weeks, which suggests that its presence in soil in the field (see above) is emphemeral and due to spores being washed down by rain.

Strains of Fusarium decemcellulare

The pigment produced by all strains develops more slowly on an alkaline (pH8.8) medium than on an acid one.

SOIL FERTILITY

Effect of amounts and combinations of major nutrients

(i) As was stated in Quarterly Report No. 43, the remaining fertilizer experiments on seedlings have been terminated because of fertilizer "placement damage" inflicted at the beginning of the experiments. Any information on responses to fertilizer application which have, or might eventually, become apparent would be quite unreliable because such responses would merely reflect the difference between an unknown amount of placement damage and a response which emerged despite the damage.

(ii) The existing NPK experiments on Upper Amazon cacao, with the exception of the one at Bunso, cannot yield information on fertilizer responses until a new method of yield recording has been introduced.

The effects of major nutrients from different sources

The 'types of P' and 'types of N' experiments will be discontinued because of the difficulty of recording yields at these sites. In addition, the need for this type of experiment is not of paramount importance at the present stage of the investigations.

VEGETATIVE PROPAGATION

The routine propagation of selected cacao types, as cuttings, for distribution to the Departments of Agriculture in the Gold Coast and Nigeria continues. The cuttings are

flown to Nigeria and for the journey are packed with their roots, which are undisturbed as far as possible, placed in polythene bags. All the cuttings are then wrapped in polythene and packed in a wooden box. Approximately 90% of these cuttings survive and are successfully established.

Baskets dipped in a 3% pentachlorophenol solution have been used in an attempt to prevent them from rotting. This scheme has been abandoned owing to the toxic effect of this chemical on leaves touching the coated basket. Solignum-dipped baskets were also tried but resulted in a lower percentage of the cuttings rooting.

A factorial experiment has been started to investigate the effect of the addition of N,P,K and Mg to the soil on the rooting and subsequent growth of cuttings. The P, K and Mg were added to the potting soil before the cuttings were set; the N will be applied as urea in liquid form after the cuttings have been hardened off, and thereafter at monthly intervals.

PLANT BREEDING AND SELECTION

Felling and poisoning of shade trees has been continued, and in the Trinidad Introductions area in particular the shade is being drastically reduced. Felling of shade trees in the clonal trials has resulted in a lot of damage to the cacao, so in an attempt to reduce damage in the future, the trees are first being poisoned, and will not be felled until dead.

The Colombian Introductions are establishing themselves well, and one plant is bearing a young cherelle.

The development of the third clonal trial continues well; measurements of the diameter of the stem, the length and breadth of the largest leaf, and counts of the number of leaves have been taken for each plant. These will give an indication of the sizes of the plants soon after planting, which may prove to be correlated with their future performance. Measurements of the stem diameter are being made at half-yearly or yearly intervals in the 8th and 9th progeny trials and in the trials on the Department of Agriculture cocoa stations.

Regular yield recording in the 2nd progeny trial is being discontinued from the end of this season, and the determination of wet weight of cocoa for this trial has already been discontinued. This trial was planted in 1942, and the evaluation of the selections in it is now reasonably complete.

For the determination of long-term trends it will be sufficient to take yield records for a year at intervals of three to four years.

Records are to be taken on the better selections in the 1st progeny trial this coming season; regular recording of this trial was discontinued in 1954.

Fermentations of some of the better-yielding selections in the 8th and 9th progeny trials have been carried out; the cocoa is to be submitted for quality-testing. These selections include WEB2 in the 9th progeny trial (Planted as seedlings May, 1954), which is growing with remarkable vigour.

Planting of pods from the 1st albino plot has been discontinued as it seems unlikely that it can give much more information, and the nursery space is needed for pods from the larger 2nd albino plot. The difference between the outer rows of trees and the inner group in the proportion of pods resulting from pollinations from outside the plot, which was mentioned in Quarterly Report No. 43, has proved not to be statistically significant.

The E.1 plot at Bunso is now mostly in bearing, and on the basis of pod-form a number of rogues have been detected in addition to the two previously known. These probably contribute significantly to the setting that is taking place throughout this 5-acre plot of a self-incompatible clone. An experiment is being planned in conjunction with the Entomology Division to seek to determine the range of pollination from one of these rogues after removal of the others.

COCOA PREPARATION AND QUALITY

Fermentation and chemical constitution

The results of experimental work carried out during the 1955 main crop showed that the fermentation of cocoa does not take place uniformly throughout the mass and that the changes in the bean, associated with fermentation, becomes evident more quickly at the surface than at other parts of the bean mass.

A series of experiments has been carried out on the 1956 crop to determine to what extent the fermentation rate varies between the top and centre of the bean mass. Samples were withdrawn every 24 hours and quantitative estimation of the polyphenols made. The pH of the pulp and cotyledons was determined and sections of the cotyledons examined microscopically to determine when the polyphenols had diffused out of the storage cells. Germination tests were made on

hundred-bean samples and the temperature through the bean mass recorded at regular intervals.

The turning frequency was varied to include treatment on the second and fourth days, the third day and no turning in order to find if mixing does, in fact, have any effect on uniformity of the end product.

The samples withdrawn from the top and centre every 24 hours were sun-dried, roasted and made into chocolate; polyphenol determinations are being made on the dried cotyledons.

The results so far show quite clearly that the surface beans are killed more quickly than those at the centre and that cocoa aroma and flavour are developed within 24 hours of the death of the bean. Destruction of the anthocyanins is also more rapid in the surface layer. Further aspects of this work cannot yet be commented on, but a full report should appear in subsequent Quarterly Reports.

Studies on Cacao enzymes

To obtain a better understanding of the chemical changes which take place during fermentation a study is being made of the properties of some of the enzymes of beans. It has been suggested that during the early phase of fermentation some of the preliminary stages of germination take place and that among these will be the development of enzymes such as cellulase and 'pectinase' which are able to break down cell-wall polysaccharides. These enzymes will cause disintegration of the walls of the polyphenol storage cells thus exposing their contents to the action of enzymes present in the other cells. This suggestion pre-supposes firstly, that the cell walls are actually broken down and secondly, that cell wall disintegrating enzymes are, in fact, present. Evidence on the first point is being sought by an anatomical investigation of beans after various times of fermentation. Acetone-powders of undried fresh, germinated and fermented beans are being used for determining enzyme levels.

Pectin-esterase activity was found in fresh and germinated beans and also in beans which had been fermented for 24 hr., but had almost disappeared after fermentation for 2 days. Polygalacturonase activity (de-esterified apple pectin as substrate) has been tested for but so far not found. Very weak cellulase activity, measured by the reduction in viscosity of sodium carboxy-methyl cellulose, has been detected in germinated beans.

The breakdown of the anthocyanins during fermentation appears not to be due to oxidase action and β-glycosidases have been suggested as the enzymes responsible. β-glucosidase activity (Salicin as substrate) has been determined and found to survive fermentation for one day but not for two. Although the β-glucosidase which hydrolyzes salicin may also break down the anthocyanins it seems likely that specific anthocyanases may be necessary.

INSECTICIDE STUDIES

Joint W.A.C.R.I. - Department of Agriculture Experiments

1. Preliminary experiments to control ants in contact areas around swollen shoot outbreaks.

These experiments, noted in Quarterly Report No. 43, were designed to examine the effect of an insecticide with high residual properties, dieldrin, on ant activity.

On three sites, each of approximately one acre, the trunks of all trees, cacao and forest species, were sprayed with dieldrin emulsion at the rate of 1 lb. toxicant per acre.

The insecticide virtually eliminated the ant population. Reinfestation of the areas by ants was noted some five weeks after the insecticidal application, and gradually increased. However, re-establishment of ant nests and organised activity (e.g. definite columns of ants up and down tree trunks etc.) has been very slow. In one plot in which organised ant activity was observed on 36 trees at the initial survey, it was observed on only 13 trees at a resurvey carried out twenty weeks later. At a second plot organised activity was noted on 89 trees at the fifteenth weekly resurvey, compared with 231 at the initial survey. The third plot received a second application of dieldrin similar to, and nine weeks after, the first. At the last weekly survey, the eleventh since the second application was made, 17 trees with organised ant activity were noted; at the initial survey there were 156.

The effect of other rates of application, and of other insecticides is also being examined.

2. The effect of large-scale formicidal spraying on the spread of swollen shoot virus.

As a result of the success of the preliminary trials in reducing ants in mature cacao by spraying the trunks with dieldrin aqueous emulsion, the scope of this work has been enlarged. In conjunction with the Department of Agriculture, a large-scale field trial has been organised to investigate

the effect of widespread formicidal spraying, and other measures, on the spread of swollen shoot virus through cacao.

A 200-acre block in the Oyoko area has been sprayed with dieldrin emulsion at the rate of 1 lb. toxicant per acre. The operation has been carried out by the spraying teams of the Department of Agriculture using 'Motoblo' mist-sprayers. Both cacao and forest trees were treated, the spray being directed principally on to the lower 8-10 ft. of the trunks. It is proposed to respray the whole block with dieldrin after an interval of eight weeks, and at the same time a portion of the block will also be sprayed with malathion. A second similar block of 200 acres about a mile distant from the first will receive no insecticidal treatment and will serve as a control.

Pre-treatment surveys of the ant population were carried out and are now being continued at regular weekly and fortnightly intervals to assess the effect of the insecticide on these insects.

Mealybug counts are also being made. Each of the treatment areas is visited once a week when mealybug counts are made to hand height on a hundred trees. Pre-treatment counts have been completed and post-treatment records, following the first application of dieldrin, are being taken. Estimates of parasitism of Pseudococcus njalensis are being made and all other vector species are retained for identification. The presence, or absence, of tents over mealybug colonies and the number of colonies attended by ants are recorded in the field.

Whilst the shortcomings of estimations of the mealybug population based purely on counts to hand height are fully realised it has not been possible to fell complete trees, as a routine means of estimation, due to the experiments being carried out on farmers' cacao. The method employed should, however, be sufficient to reveal gross fluctuations in the population and changes in the proportion of species composing the vector population. The experiment has not been underway sufficiently long for it to be possible to comment on the fate of mealybug populations in the absence of attendant ants. The criterion of success will be the number of new outbreaks of swollen shoot disease which appear after the incubation period of the virus, in trees with latent infection at the start of the experiment, has elapsed. The records of the diseased trees removed are being maintained by the Department's staff.

Pollination Studies

(1) Entomological

Collections of insects in cacao flowers showed that the pollinating midges were more abundant than in the previous quarters.

(2) Botanical

Comparison of natural pollination and setting rates throughout the year. During October and November the numbers of flowers increased somewhat, but fell again in December. Setting followed the same pattern, the October-November peaks being the highest yet recorded; pollinations were correspondingly frequent, as many as 80-90% of the flowers being pollinated in the first 24 hours after opening.

THE PHYSIOLOGY OF NUTRITION, GROWTH AND FRUITING

Root growth

The seedlings from seed sown in June 1955 on the Koforidua series soil are growing rapidly, and monthly samples of 4 plants are being taken to follow the growth of the different parts of the plant.

Many of the original Amelonado seedlings on the Akwadum series soil were replaced in July 1956 by Upper Amazon seedlings, as these are more vigorous and were thought to be more likely to survive the poor soil conditions.

Investigation of cherelle wilt

Effect of increased setting on yield

The latest results of the experiment on the effect of increased setting on yield (see earlier Quarterly Reports) are as follows:-

	Hand pollination (20 trees)	Natural pollination (20 trees)
Pods set	4075	3431
Pods wilted	3308	2725
% wilted	81.3%	79.5%
Pods harvested	631	615
Pods remaining on trees (26.12.56)	136	91

The pods remaining on the tree are all likely to mature. The hand pollination has made relatively little difference to the number of pods harvested, and in the final analysis it is unlikely that the difference will be significant.

In another experiment all flowers and cherelles were removed until after the April flush. Very few cherelles survive this flush period. The following table shows the number of pods harvested over the course of the year from the 48 trees in the experiment:-

Treatment	Time of harvesting						Total	Pods Remaining on trees
	June-Aug	Sept	October		Nov	Dec		
			1st half	2nd half				
Flowers removed	0	1	27	60	86	40	214	366
Control	13	16	73	40	51	14	207	399

The treatment has delayed the harvest but has not greatly reduced it. This suggests that the total yield of a tree during a season is not greatly affected by the number of cherelles which survive through this flush period.

In both the above experiments the yields have not been greatly affected by the treatments given. This suggests that some other factor as yet unidentified is limiting yield. The discovery of the nature of this factor and how it could be controlled would be a distinct step forward in our efforts to increase yield.

Frequency of harvesting

In the early part of the season, when relatively few pods were harvested, there were indications that weekly harvesting resulted in a greater total harvest than monthly harvesting. This effect did not persist, however, and the totals up to 9th December are: weekly harvesting 2201; monthly harvesting 2227.

Experiments with radioactive phosphorus

The experiment to follow the uptake of P^{32} from the soil over the course of a year continues. The method involves assessing the radioactivity throughout the tree after a 10 day period of uptake. Sample disks are taken from the leaves, branches and trunk and tested for activity. Very high amounts of P^{32} have been found in rapidly elongating chupons, sprouting buds and young cherelles, but it is too early to discuss the implications of these results.

Nutrient uptake by cacao in water culture

Work is starting on a study of the uptake of nutrients from water-culture solutions. It is hoped to grow cacao in these solutions to the stage of fruiting trees. This work will be carried out in close association with the Soil Scientist in order to compare results with those obtained in the field.

STUDY OF THE EFFECTS OF SHADE IN CACAO CULTIVATION

No progress report has appeared under this heading since July - September 1955 (Quarterly Report No. 39). In future any field experiments which are directly linked with soil fertility studies and have hitherto come under Section 13 will be included in Section 6.

Effect of forest litter removal

This experiment has been discontinued because the top-soil at the site was found to be extremely variable. In addition, the fortnightly removal of forest litter was found to be unsatisfactory because earthworn activity resulted in the burying of some litter before it was removed.

ADDITIONAL INVESTIGATIONS

Entomological aspects of personnel health: Transmission of 'Filaria' parasites (Acanthocheilonema streptocerca) by Culicoides spp.

Infection rates: Examination of nearly 3,000 flies from various sites in the cacao, the Residential Area and W.A.C.R.I. Village provides the following estimate of infection rates:-

Total flies caught	2,850
Flies with infective stage	3
Flies with other stages	7
Rate of infection (infective stages only)	0.11%
Rate of infection (all stages)	0.35%

An attempt is being made to correlate the number of biting females with various climatic factors.

An investigation into the breeding sites of sandflies has been commenced, in the hope of being able to reduce the numbers of this pest by localised spraying of the most common sites.

IBADAN SUB-STATION

VIRUS RESEARCH

Coppicing experiments at I.N.A. Farm

Routine recording and harvesting has continued on the various coppicing experiments at the Ibadan Native Administration farm. Several of the one-acre plots have now been coppiced for more than two years and on all of these some of the chupons have begun to bear pods. Pods are not produced during the first year after coppicing and yield during the second has been found to vary considerably, depending upon

spacing and size of the trees and the time of year when coppiced. Regeneration from closely spaced trees at 8' x 8' is in general more satisfactory than from those more widely spaced, and most of the pods have been produced on chupons from trees which were greater than 16" in girth at the time they were cut back. From the outset, new growth from coppiced stumps appears to show the same periodicity of bearing as mature, intact trees and consequently the maximum yield during the second year after coppicing is obtained when a main-crop season falls towards the end of this period. Records for the various plots treated in 1954 illustrate this point, as few trees coppiced in May of that year produced pods during their second year of growth, whereas plots coppiced in November produced an average of one pod per stand.

A sample of thirty mature trees of uniform girth and condition was coppiced in January 1956. Comparable samples were coppiced at intervals of three months and detailed observations were carried out on the appearance and rate of growth of chupons from the 120 coppiced stumps. The results obtained agreed closely with those from the similar, but more detailed, experiment carried out in 1955. It was again found that trees coppiced in July produced fewer chupons and at a slower rate than trees coppiced during the early or late months of the rainy season, or during the dry season.

The control of swollen-shoot disease in Nigeria

The revised method of treating swollen-shoot outbreaks in Nigeria which was detailed in Quarterly Report No. 43 has now been in operation for six months. The survey parties are finding that the less drastic treatments can be applied more quickly and result in a great reduction in the number of trees cut out, compared with the previous system of treating all out-breaks to a distance of 30 yards from the nearest infection. Information now being collected should indicate whether the revised method is any less efficient than the previous one in controlling the spread of virus.

Symptom expression and virus availability in mature trees

From time to time field officers of the Western Region Department of Agriculture report that trees originally showing clear symptoms of virus infection have, on one or more subsequent dates, been found quite symptomless. Recent observations have again drawn attention to this problem which is an important one affecting the efficiency of all control operations. An outbreak was reported in 1955, south of the

area of mass infection, and all the known infected trees were marked after a thorough inspection carried out in May of that year. Cutting-out operations were delayed and when the area was reinspected, the number of known infections had increased from 1,324 to 3,332 in the 19-month interval. Some 246 of the trees found with symptoms in 1955 are now reported to be symptomless. This is a surprisingly high percentage, but the estimate is a reliable one as on each occasion the inspections were carried out under the close supervision of a senior officer. Furthermore, the same phenomenon has been noted at the special observation plot at Koroboto, where up to 10% of the known infected trees were symptomless at any one of the monthly inspections. It appears, from work carried out here and elsewhere, that certain trees are tolerant of infection or are infected with very mild strains of virus and, therefore, show symptoms for a short time only before passing into a symptomless phase of variable duration. A tree which stops producing symptoms even temporarily is soon likely to become symptomless under Nigerian conditions, as leaves commonly persist only from one flush to the next and, as a result of capsid attack, branches are often damaged to such an extent that swellings become unrecognisable.

To assess the danger of latently infected trees to surrounding healthy cacao it is necessary to determine the availability of virus to mealybugs feeding on them. This has been done by sampling trees in different phases of symptom expression and comparing the transmission rates obtained when mealybugs were fed on the tissues and then transferred to dissected beans. Using a standardised procedure, the best results were obtained on transferring mealybugs from trees with severe and extensive symptoms. Virus was less readily available to mealybugs feeding on material from trees with very mild symptoms or from those in a symptomless phase of infection, and results from the latter were very erratic. Tests on some of the symptomless trees were all negative, whereas others proved to be a good source of virus, though it was usually observed that these produced new leaves with symptoms soon after the experimental material had been collected from them.

Mealybugs feeding on succulent stems proved more likely to infect beans than those feeding on hardened tissue out of flush. In a subsidiary experiment pods proved to be a poor source of virus compared with succulent or even hardened

stem tissue.

In carrying out experiments on virus availability it was found necessary, because of differences in the skill of operators, to arrange that each man handled an equal number of specimens from each group of trees.

The size, composition and behaviour of the mealybug population on infected trees obviously affect their potency as sources of virus, and it seems from this work on virus availability that other important factors are the severity of symptoms on infected trees and their flushing behaviour. Similar results were obtained by Posnette and Robertson (Ann.appl.Biol., 37, p.363, 1950) with the Mampong virus in the Gold Coast.

CAPSID STUDIES

Chemical control of Capsids

Capsid damage to the cacao canopy increased on all untreated plots during the quarter. Differences between these and the treated plots, which had previously been negligible, began to show up during the last week in September. There was then little apparent difference between the treatments themselves, all of them giving good control. Later, a marked increase in damage was observed on untreated plots and it was noticeable that treatments C and F (see table below) were not giving such good control as the others. This is substantiated by records taken from the marked trees as illustrated below:-

	Treatment	% damage * (chupon and fan)
A.	2 oz.gamma-BHC/acre low volume every 4 weeks from Aug. to Feb. inclusive	1.28
B.	4 oz.gamma-BHC/acre low volume every 4 weeks from Aug. to Feb. inclusive	1.17
C.	4 oz.gamma-BHC/acre low volume every 8 weeks from Aug. to Feb. inclusive	7.32
D.	4 oz.gamma-BHC/acre high volume every 4 weeks from Aug. to Feb. inclusive	1.71
E.	4 oz.gamma-BHC/acre low volume in Aug. and Sept. followed by high volume applications where damage occurs	2.40
F.	4 oz. gamma-BHC/acre hand dusting in Aug. and Sept. followed by hand-dusting applications where damage occurs	7.95
G.	No treatment	105.5

* These figures are calculated by taking the numerical evaluation of capsid damage to chupon and fan growth in November as a percentage of that recorded during the first assessment in July.

Treatment D, which is applied by the 'Four Oaks' pneumatic knapsack sprayer has given excellent control to date. This equipment cannot, however, fully cover mature cacao trees because the spray only reaches a height of about ten feet.

It is thought that capsid counting is now of little value, as most of their feeding is taking place in the canopy and they are, therefore, beyond the reach of the collectors. Figures do show, however, that: (1) the peak population on untreated plots was reached in September, and there has since been a gradual decline; (2) the third insecticidal application reduced the population on all treated plots to a low level.

Harvesting records seem to indicate that treatment of mature cacao severely damaged by capsids may well have an effect on yield in the first year of spraying. Yields recorded for untreated plots during the last two months are the lowest, but at present no definite conclusion can be drawn from the figures as they have not been subjected to statistical analysis.

Control of Ants associated with Capsid control

To obtain some indication of the effect of gammalin on ants and, therefore, indirectly on mealybugs, ant counts were started in November. Counts (of black species only) are taken from 100 marked trees on each of the 63 plots during normal monthly counting. Numerical evaluation of populations will be as follows: a nest or nests seen on the tree - 3 points; a column or columns of ants on the tree - 2 points; single ants scattered about the tree - 1 point.